

Evidence that the N-Terminal Region of the *Vibrio fischeri* LuxR Protein Constitutes an Autoinducer-Binding Domain

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The *Vibrio fischeri* luminescence genes are regulated by the LuxR protein and an *N*-acyl homoserine lactone compound termed the autoinducer. The C-terminal one-third of LuxR contains a domain that can interact with the transcription complex and activate the luminescence genes. On the basis of limited evidence it has been suggested that the N-terminal two-thirds of LuxR constitutes a domain that serves to bind the autoinducer. We show that tritium-labeled autoinducer binds to *Escherichia coli* cells in which LuxR is overexpressed. We also show that tritium-labeled autoinducer binds to *E. coli* cells in which truncated LuxR proteins missing portions of the C-terminal domain are expressed but does not bind to *E. coli* cells in which truncated LuxR proteins missing portions of the N-terminal region are expressed. Our results provide evidence that the autoinducer binds to LuxR and that in *E. coli* the N-terminal two-thirds of LuxR can fold into a polypeptide capable of binding the autoinducer in the absence of the C-terminal domain.

The *Vibrio fischeri* LuxR protein is a member of a family of transcription factors involved in a phenomenon termed quorum sensing and response. Members of the LuxR family have been found in a number of gram-negative bacteria that enter into associations with eukaryotic hosts (11, 23). In quorum sensing the bacteria produce an *N*-acyl homoserine lactone, the autoinducer. The *V. fischeri* autoinducer is *N*-(3-oxohexanoyl)homoserine lactone (9). Cells are freely permeable to this autoinducer, which accumulates in the culture medium during growth (15). When the autoinducer reaches a sufficient concentration (intracellular and extracellular concentrations are equivalent), LuxR activates transcription of the luminescence (*lux*) genes (8, 10, 17). Thus, LuxR and the autoinducer are components of a chemical communication system that enables *V. fischeri* to sense its own cell density. *V. fischeri* occurs at low cell densities in seawater and at high cell densities as the specific bacterial symbiont in light organs of certain marine animals (for recent reviews, see references 7 and 19). The autoinducer accumulates in the high-cell-density light organ environment, and this leads to the expression of the *lux* genes.

More is known about the structure and function of LuxR than about any other transcriptional regulator in the LuxR family. The C-terminal one-third of this 250-amino-acid protein constitutes a domain capable of binding *lux* regulatory DNA and activating transcription of the luminescence genes (4, 5, 22). Genetic evidence suggests that the autoinducer binds directly to LuxR (13, 20, 21). In addition, Adar and Ulitzur (2) recently have shown that the autoinducer binds to *Escherichia coli* in which LuxR is overproduced. Hsp60 stimulates the processing of LuxR into an active conformation in *E. coli* (1, 6), and autoinducer binding to LuxR-containing *E. coli* is dependent on overexpression of Hsp60 (2). In *E. coli* expressing LuxR proteins with specific amino acid substitutions in the central region of LuxR (between residues 79 and 127), induction of the luminescence system occurs only in the pres-

ence of very high concentrations of the autoinducer (20, 21). This together with the finding that the C-terminal one-third of the LuxR constitutes an autoinducer-independent activator of *lux* gene transcription (4) has led to a model in which the N-terminal two-thirds of LuxR constitutes an autoinducer-binding, regulator domain that inhibits the activity of the C-terminal domain in the absence of the autoinducer (4, 11). However, there is no direct evidence that the N-terminal region of LuxR can fold into a domain capable of autoinducer binding. We report a refinement of the recently described autoinducer-binding assay (2), and we present evidence obtained by using this assay that the N-terminal two-thirds of LuxR bind autoinducer.

The autoinducer-binding assay described by Adar and Ulitzur (2) involves incubation of *E. coli* with the autoinducer followed by washing to remove unbound or loosely bound autoinducer from the cells. Tightly bound autoinducer is then released by heat treatment. The released autoinducer is measured by a quantitative bioassay. Making use of ³H-autoinducer synthesized previously (14), we have developed a modification of the autoinducer-binding assay in which autoinducer that is tightly bound to *E. coli* cells can be measured directly without releasing this autoinducer with a heat treatment or determining its concentration by means of a bioassay.

For ³H-autoinducer binding experiments, cultures of *E. coli* XL-1 Blue (3) containing various plasmids (Table 1) were grown at 30°C in Luria broth with 1 mM isopropyl-β-D-thiogalactoside to activate the *ptac*-controlled *luxR* genes and the *plac*-controlled *groESL* genes (6). The appropriate antibiotics were added for plasmid maintenance (4). The initial culture density (optical density at 600 nm) was 0.05 to 0.10. When the culture density reached an optical density at 600 nm of 0.3, 1-ml volumes were transferred to microcentrifuge tubes, ³H-autoinducer (25 Ci/mmol) was added (29.2 nmol in 30 μl of deionized water unless otherwise specified), and the mixtures were incubated at 25°C for 10 min. This incubation was followed by centrifugation at 16,000 × *g* for 4 min. The cell pellets were then washed and centrifuged twice in phosphate-buffered saline and finally resuspended in 30 μl of phosphate-buffered saline. The radioactivity in each 30-μl sample was determined by standard scintillation counting procedures. The amount of

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TABLE 1. Plasmids used

Plasmid	Relevant characteristics ^a	Reference
pHK724	<i>ptac</i> -controlled synthesis of full length LuxR	16
pSC005	<i>ptac</i> -controlled synthesis of a LuxR-deletion, Δ2-5	4
pSC010	<i>ptac</i> -controlled synthesis of LuxR Δ2-10	4
pSC058	<i>ptac</i> -controlled synthesis of LuxR Δ2-58	4
pSC127	<i>ptac</i> -controlled synthesis of LuxR Δ2-127	4
pSC130	<i>ptac</i> -controlled synthesis of LuxR Δ2-130	4
pSC138	<i>ptac</i> -controlled synthesis of LuxR Δ2-138	4
pSC315	<i>ptac</i> -controlled synthesis of LuxR Δ236-250	5
pSC335	<i>ptac</i> -controlled synthesis of LuxR Δ216-250	5
pSC350	<i>ptac</i> -controlled synthesis of LuxR Δ201-250	5
pSC357	<i>ptac</i> -controlled synthesis of LuxR Δ184-250	5
pSC389	<i>ptac</i> -controlled synthesis of LuxR Δ162-250	5
pGroESL	<i>plac</i> -controlled synthesis of Hsp60	12

^a pGroESL is a P15A replicon with a chloramphenicol resistance marker. All of the other plasmids are ColE1 replicons with ampicillin resistance markers.

the autoinducer retained by the cells was calculated from the radioactivity of the sample and the specific activity of the autoinducer.

Although we used an *E. coli* strain, and vectors to provide LuxR and Hsp60 that were different from those used by Adar and Ulitzur (2), we obtained results with our modified autoinducer-binding assay that were remarkably similar to those reported previously. When both LuxR and Hsp60 were overexpressed, binding of the autoinducer was detected. The amount of binding was dependent on the external autoinducer concentration (up to 100 to 200 nM) (Fig. 1) (2), and the dose-response was essentially identical to that reported by Adar and Ulitzur (2). It is interesting that saturation of autoinducer binding reflects the level of the autoinducer required to saturate the luminescence induction response (100 to 200 nM; for an example, see reference 15). Autoinducer binding to *E. coli* cells required *luxR* (Table 2). In our experiments, the autoinducer did bind to cells without the Hsp60 expression vector, pGroESL; however, the amount of the autoinducer bound by the cells was about 10-fold less than the quantity that bound to

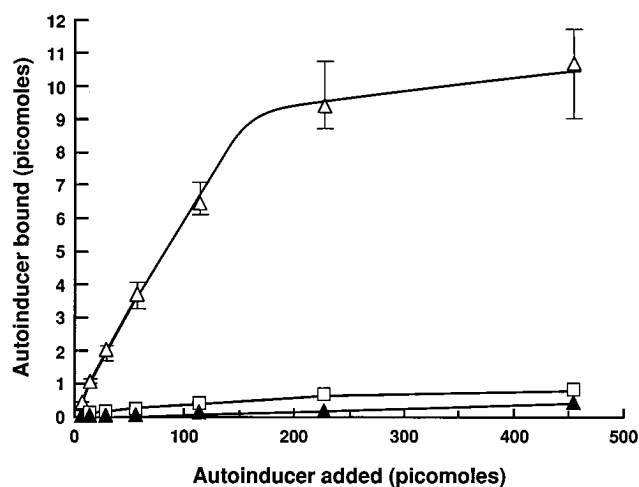


FIG. 1. Dose-response for autoinducer binding to *E. coli* containing pGroESL (▲), *E. coli* containing pGroESL and pSC005 (□), or *E. coli* containing pGroESL and pSC010 (△). The bars indicate the ranges in two or three independent experiments. The ranges for experiments with *E. coli*(pGroESL) and *E. coli*(pGroESL) are not shown because in all cases they were smaller than the size of the symbols. The conditions for the ³H-autoinducer-binding assay are described in the text.

TABLE 2. ³H-autoinducer binding to *E. coli* containing *luxR* and *groE* plasmids

Plasmid(s)	LuxR product ^a	Amt of ³ H-autoinducer bound (pmol) ^b
pGroESL	None	0.03 (0.03–0.04)
pHK724	Wild type	0.04 (0.036–0.045)
pHK724, pGroESL	Wild type	0.23 (0.227–0.229)
pSC315, pGroESL	Δ236-250	1.9 (1.3–2.4)
pSC335, pGroESL	Δ216-250	2.1 (2.0–2.1)
pSC350, pGroESL	Δ201-250	2.1 (1.85–2.3)
pSC357, pGroESL	Δ194-250	2.6 (2.4–2.8)
pSC357	Δ194-250	2.8 (2.7–3.0)
pSC389, pGroESL	Δ162-250	0.12 (0.10–0.13)
pSC005, pGroESL	Δ2-5	0.04 (0.04–0.05)
pSC010, pGroESL	Δ2-10	2.5 (2.5–2.7)
pSC058, pGroESL	Δ2-58	1.9 (1.8–2.1)
pSC127, pGroESL	Δ2-127	0.01 (0.01–0.01)
pSC130, pGroESL	Δ2-130	0.01 (0.01–0.01)
pSC138, pGroESL	Δ2-138	0.04 (0.04–0.05)
		0.02 (0.02–0.03)

^a For pSC plasmid-containing *E. coli*, the amino acid residues deleted from the LuxR polypeptides are indicated. Western immunoblotting as described previously (4, 21) was performed to show that the appropriate-size LuxR polypeptide was overexpressed in all of the *E. coli* transformants listed.

^b Values are means of two to five independent experiments. The numbers in parentheses are the ranges of values.

cells with both the LuxR and the Hsp60 expression vectors. Nevertheless, LuxR-containing cells without the Hsp60 expression vector bound considerably more autoinducer than cells without a *luxR* gene (Table 2). Adar and Ulitzur (2) were unable to detect any autoinducer binding above background unless the cells contained an Hsp60 expression vector. In any case, our results confirm the conclusions that autoinducer binding depends on LuxR and that Hsp60 assists in forming LuxR into an active autoinducer-binding protein.

To gain an appreciation of the region of LuxR involved in autoinducer binding and to test the hypothesis that there is an N-terminal autoinducer-binding domain, we measured autoinducer binding to *E. coli* containing a series of *luxR* deletion plasmids (Table 2). *E. coli*(pGroESL) containing plasmids that direct the synthesis of truncated LuxR proteins missing the C-terminal 15, 35, 50, and 57 amino acids bound the autoinducer equally as well as *E. coli* containing a plasmid that directs the synthesis of full-length LuxR. *E. coli*(pGroESL) containing a plasmid directing the synthesis of a truncated LuxR protein missing the C-terminal 89 amino acids did not bind more autoinducer than *E. coli* without *luxR*. These results are consistent with the hypothesis that autoinducer binding is to the N-terminal region of LuxR and that there is an N-terminal domain of LuxR extending to a site between residue 161 and residue 193. Apparently, this domain can fold into a polypeptide capable of binding the autoinducer in *E. coli*.

E. coli expressing the truncated LuxR protein missing the C-terminal 57 amino acids bound significantly less autoinducer without the Hsp60 expression vector than with this vector (Table 2). This indicates that the N-terminal autoinducer-binding domain requires Hsp60 for proper folding or some other reason. Whether the C-terminal DNA-binding domain also requires Hsp60 remains to be determined.

It was shown previously that in *E. coli*, LuxR polypeptides with small deletions of the first 10 to 20 amino acids in the N terminus show an autoinducer-dependent ability to activate luminescence gene transcription (4). Proteins with larger de-

letions show little ability to activate luminescence, and the autoinducer does not affect transcriptional activation by these proteins. Either the low-level activity proteins with large deletions ($\Delta 2-58$, $\Delta 2-127$, $\Delta 2-130$, $\Delta 2-138$) have lost the ability to bind the autoinducer, or autoinducer binding to these proteins does not relieve the inhibitory effect of the N-terminal domain on the C-terminal transcriptional activator domain (4). Our evidence shows that these proteins did not retain autoinducer-binding activity in *E. coli* (Table 2). As expected, *E. coli* expressing LuxR proteins with small deletions ($\Delta 2-5$ and $\Delta 2-10$) showed autoinducer binding comparable to that of *E. coli* expressing wild-type LuxR (Table 2). Several plasmids with *luxR* missense mutations that map to the N-terminal domain of LuxR have been characterized (20, 21). These plasmids encode LuxR polypeptides with single amino acid substitutions that are either inactive or active only at high autoinducer concentrations. *E. coli* XL1-Blue(pGroESL) containing one such plasmid, pDV751, which codes for a LuxR polypeptide containing a Gly-121-to-Arg substitution, does not bind ^3H -autoinducer at levels higher than background (18).

In summary, we show that radioactive autoinducer binds to *E. coli* cells expressing the *V. fischeri* LuxR protein. Binding is enhanced in cells containing the Hsp60 expression vector, pGroESL (Table 2). This confirms the report of Adar and Ulitzur (2), provides evidence that the autoinducer binds to LuxR directly (2, 13, 20, 21), and supports the conclusion that Hsp60 assists in the formation of active LuxR (1, 2, 6). Our results also provide strong evidence for the hypothesis that the N-terminal two-thirds of LuxR constitutes a domain that can function independently of the C-terminal transcriptional activator domain. The data presented in Table 2 indicate that this N-terminal domain extends from somewhere between residue 10 and residue 58 to a point between residue 162 and residue 193. These results are entirely consistent with our previous conclusion that the C-terminal domain begins at a point located between residue 162 and residue 182 (4). A simple ^3H -autoinducer-binding assay will be useful in attempts to further study the mechanism of the autoinducer-LuxR interaction. We hope that this approach will be generally applicable to studies of the binding of structurally related autoinducers from other bacteria to the LuxR homologs of these bacteria.

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